

MORPHOLOGICAL AND ELECTROPHORETIC VARIATION  
AMONG THE FOUR FLORAL COLOR MORPHS OF  
*CLARKIA AMOENA* VAR. *PACIFICA* (ONAGRACEAE)

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ABSTRACT

We examined morphological and electrophoretic variation in the four floral color morphs of *Clarkia amoena* var. *pacifica* (Peck), a plant native to grassy coastal headlands of Oregon. Mean values of morphological characters such as petal length and width, leaf length and width, fruit length, and plant height differed between the white color morph and the other three, with the white color morph being consistently larger (except for leaf width). Electrophoretic analysis at 24 loci revealed higher heterozygote frequencies and greater numbers of effective alleles in the white color morph. Nei's measure of genetic identity showed high similarity among the color variants but the white floral morph was less similar to the other morphs than they were to each other ( $\bar{I} = 0.94$  versus  $\bar{I} = 0.99$ ). Since the white morph occurs in a more isolated subpopulation at Cascade Head where the other three morphs occur sympatrically, its divergence from them in morphology and isozyme patterns might eventually lead to population differentiation and speciation.

Rapid evolutionary change often takes place in small, isolated populations and studies of such populations can lend understanding to evolutionary processes (Waser et al. 1982; Karron 1987). Examples of progenitor/derivative pairs within the genus *Clarkia* have been exceptional in this regard, providing evidence that rapid and recent speciation has occurred numerous times (Lewis 1953, 1955, 1966; Moore and Lewis 1965; Smith-Huerta 1986).

In *Clarkia*, over forty species of annuals are distributed primarily throughout the western United States. These species generally occur in small, discontinuous populations of a few to several hundred or even a thousand individuals (Smith-Huerta 1986). The genus *Clarkia* has been widely studied to determine the evolutionary relationships among various species (Lewis and Lewis 1955; Lewis and Raven 1958).

*Clarkia amoena* includes three varieties which range from central California northward to Vancouver Island (Hitchcock and Cronquist 1973). This study focuses on *Clarkia amoena* var. *pacifica*, a small flowered variety of the species which only occurs on grassy coastal headlands of Oregon. We are particularly interested in the possible relationships between petal color variation, morphology, and the genetics of populations occurring in a coastal prairie at Cascade Head,

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Oregon. In addition, we address the potential implications of floral color variation for the evolution of *Clarkia* in small localized populations.

#### MATERIALS AND METHODS

*Study site.* Initially, we surveyed the northern and central coast of Oregon for populations of *Clarkia amoena* var. *pacifica*. We found the variety in only two locations, Cascade Head and Cape Lookout. At Cape Lookout, we observed only one color morph (purple). Therefore, we selected Cascade Head, where four color morphs occurred, as our study site. At Cascade Head (a Nature Conservancy Preserve near Lincoln City, OR) we studied two populations of *Clarkia amoena* var. *pacifica*. One population, located in the "pinnacle" region of the headland, contained three of the four color morphs: purple spotted, white spotted and purple ("spotted" morphs contained an additional magenta spot in the center of each white or purple petal). The second population, located inland approximately 0.8 km, occurred adjacent to The Nature Conservancy trail and contained only the white morph.

*Electrophoresis.* To assess genetic differences among the color morphs of *Clarkia amoena* var. *pacifica*, we examined 13 enzymes using starch gel electrophoresis. We collected leaf samples from twenty individuals of each color morph from Cascade Head, and extracted enzymes from fresh material with a Tris-HCl grinding buffer (Soltis et al. 1983). Starch concentration in the gels was 12%.

Four buffer systems produced resolvable enzyme patterns. We used a lithium-borate buffer system (Werth 1985) for alcohol dehydrogenase (ADH), aspartate aminotransferase (AAT), esterase (EST), isocitrate dehydrogenase (IDH) and phosphoglucoisomerase (PGI). A tris-citrate buffer system (Soltis et al. 1983) resolved acotinase (ACO), isocitrate dehydrogenase (IDH) and phosphoglucomutase (PGM). A histidine-citrate buffer system (Stuber et al. 1977) resolved glyceraldehyde-3-phosphate (G3PDH-NAD and G3PDH-NADP forms) and 6-phosphogluconate dehydrogenase (6PGD). Finally, we used a citrate-morpholine buffer system (Wendel and Stuber 1984) for malate dehydrogenase (MDH) and shikimic dehydrogenase (SKD).

Staining followed standard recipes: AAT and ADH were stained according to Cardy et al. 1983; ACO, G3PDH, IDH, MDH, PGI, PGM, and 6PGD are provided in Shaw and Prasad 1970; EST and SKD were stained according to Soltis et al. 1983.

We made the following calculations for each color morph: the percentage of loci that are polymorphic per population (P), expected heterozygosity ( $H_{exp}$ ), observed heterozygosity ( $H_{obs}$ ) and effective number of alleles ( $A_e$ ). Nei's genetic identities (Nei 1972) were calculated for all pairwise comparisons of the color morphs. Using

GENESTAT (Lewis and Whitkus 1989) we calculated measures of genetic diversity within and among the color morphs ( $H_T$ ,  $H_S$ ,  $G_{ST}$ ).

*Morphology.* To examine possible morphological differences among the color variants within natural populations, we randomly selected 50 plants of each color morph in June 1993. We measured several morphological characters on each plant: petal length and maximum width (of first or second flower to open), leaf length and maximum width (of leaf located at the base of the flower measured), and plant height (distance from the base to the tallest bud). We also measured fruit length at maturity on ten plants of each color morph.

We established a common garden to determine whether the morphological variation we found at Cascade Head would be maintained in a common environment. We grew forty plants (12 purple spotted, 16 purple, and 12 white) in the Martha Springer Botanical Garden at Willamette University during the summer of 1993 from seeds that we collected at Cascade Head during the summer of 1992. We pooled five seeds from twenty individuals of each color morph. We germinated these seeds in the greenhouse and later transplanted them to the garden. We randomly transplanted individuals into two grids ( $4 \times 5$  plants) and spaced plants 30 cm apart to prevent effects of competition. Although all color morphs successfully germinated, mortality rates were high during the seedling stage and no white spotted plants survived to maturity, despite three separate attempts. We measured petal length, petal width, leaf length and leaf width on the plants grown in the garden. We examined differences among the means using analysis of variance. If the ANOVA showed significant variation among means, we used the Tukey test to identify the sets of means that were statistically distinguishable (Zar 1984).

## RESULTS

*Electrophoresis.* The 13 enzymes encoded 24 scorable loci. Sixteen loci were monomorphic (AAT-1, ACO-1, ADH, EST-1, G3PDH-NAD-1, G3PDH-NAD-2, G3PDH-NAD-3, IDH-1, MDH-1, MDH-2, 6PGD-1, 6PGD-2, PGI-1, PGI-2, SKD-1 and SKD-2), and the eight remaining loci were polymorphic for one or more of the color morphs. In 6PGD we attributed a zone of fixed heterozygosity to a gene duplication (Odrzykoski and Gottlieb 1984).

Statistical measures of genetic variation indicate slight differences among the color morphs.  $P$ , the mean percentage of loci polymorphic per color morph was identical for the purple spotted, purple and white spotted color morphs ( $P = 45.5\%$ ) and somewhat lower for the white ( $P = 40.9\%$ ). The mean expected heterozygosity ( $H_{exp}$ ) and the mean observed heterozygosity ( $H_{obs}$ ) varied slightly among morphs and were greatest in the white color morph (Table 1). The

TABLE 1. OBSERVED HETEROZYGOSITY ( $H_{OBS}$ ), EXPECTED HETEROZYGOSITY ( $H_{EXP}$ ) AND EFFECTIVE NUMBER OF ALLELES ( $A_E$ ) FOR POLYMORPHIC LOCI. Means are across all loci. WS = White spotted, PS = Purple spotted, P = Purple, W = White.

| Locus        | $H_{obs}$ |      |      |      | $H_{exp}$ |      |      |      | $A_e$ |      |      |      |
|--------------|-----------|------|------|------|-----------|------|------|------|-------|------|------|------|
|              | WS        | PS   | P    | W    | WS        | PS   | P    | W    | WS    | PS   | P    | W    |
| Est-2        | 0.20      | 0.55 | 0.10 | 1.00 | 0.18      | 0.40 | 0.10 | 0.50 | 1.22  | 1.66 | 1.11 | 2.00 |
| Idh-2        | 0.55      | 0.40 | 0.50 | 0.60 | 0.40      | 0.32 | 0.38 | 0.42 | 1.66  | 1.47 | 1.60 | 1.72 |
| Aco-2        | 0.15      | 0.15 | 0.15 | 0.05 | 0.14      | 0.22 | 0.29 | 0.35 | 1.16  | 1.28 | 1.41 | 1.54 |
| Pgm-1        | 0.75      | 0.60 | 0.55 | 0.90 | 0.53      | 0.50 | 0.44 | 0.55 | 2.11  | 2.00 | 1.78 | 2.23 |
| Pgm-2        | 1.00      | 0.80 | 0.90 | 1.00 | 0.50      | 0.50 | 0.50 | 0.50 | 2.00  | 1.98 | 2.00 | 2.23 |
| Aat-2        | 0.11      | 1.00 | 0.56 | 0.00 | 0.48      | 0.50 | 0.40 | 0.00 | 1.91  | 2.00 | 1.67 | 1.00 |
| G3pdh-nadp-1 | 0.10      | 0.30 | 0.10 | 1.00 | 0.10      | 0.26 | 0.10 | 0.50 | 1.11  | 1.34 | 1.11 | 2.00 |
| G3pdh-nadp-2 | 0.00      | 0.00 | 0.00 | 0.00 | 0.65      | 0.68 | 0.68 | 0.75 | 2.82  | 3.13 | 3.08 | 4.00 |
| Mean         | 0.12      | 0.16 | 0.12 | 0.19 | 0.12      | 0.14 | 0.12 | 0.15 | 1.25  | 1.29 | 1.24 | 1.35 |

mean number of effective alleles varied from 1.24 in the purple morph to 1.35 in the white morph (Table 1).

Frequencies of alleles at the polymorphic loci were highly variable among the color morphs (Table 2). Only three alleles were not common to all color morphs. At AAT-2, the white color morph did

TABLE 2. ALLELE FREQUENCIES OF POLYMORPHIC LOCI FOR EACH OF THE COLOR VARIANTS OF *CLARKIA AMOENA* VAR. *PACIFICA*. Abbreviations as in Table 1.

| Locus        | Allele | PS   | WS   | P    | W    |
|--------------|--------|------|------|------|------|
| Est-2        | 1      | 0.72 | 0.88 | 0.95 | 0.50 |
|              | 2      | 0.28 | 0.12 | 0.05 | 0.50 |
| Idh-2        | 1      | 0.80 | 0.72 | 0.75 | 0.70 |
|              | 2      | 0.20 | 0.28 | 0.25 | 0.30 |
| Aco-2        | 1      | 0.12 | 0.08 | 0.18 | 0.22 |
|              | 2      | 0.88 | 0.92 | 0.82 | 0.78 |
| Pgm-1        | 1      | 0.50 | 0.58 | 0.70 | 0.08 |
|              | 2      | 0.50 | 0.38 | 0.30 | 0.55 |
|              | 3      |      | 0.05 |      | 0.38 |
| Pgm-2        | 1      | 0.55 | 0.50 | 0.50 | 0.50 |
|              | 2      | 0.45 | 0.50 | 0.50 | 0.50 |
| Aat-2        | 1      | 0.50 | 0.39 | 0.28 |      |
|              | 2      | 0.50 | 0.61 | 0.72 | 1.00 |
| G3pdh-nadp-1 | 1      | 0.82 | 0.95 | 0.95 | 0.50 |
|              | 2      | 0.18 | 0.05 | 0.05 | 0.50 |
| G3pdh-nadp-2 | 1      | 0.45 | 0.50 | 0.20 | 0.20 |
|              | 2      | 0.30 | 0.25 | 0.40 | 0.35 |
|              | 3      | 0.15 | 0.20 | 0.35 | 0.05 |
|              | 4      | 0.05 | 0.05 | 0.05 | 0.25 |
|              | 5      | 0.05 |      |      | 0.15 |

TABLE 3. NEI'S GENETIC IDENTITIES (1972) FOR THE FOUR COLOR VARIANTS OF *CLARKIA AMOENA* VAR. *PACIFICA* AT CASCADE HEAD. Abbreviations as in Table 1.

|    | WS    | P     | W     |
|----|-------|-------|-------|
| PS | 0.995 |       | 0.983 |
| WS |       | 0.989 | 0.938 |
| P  |       |       | 0.937 |

not express allele 1. At G3PDH-NADP-2, both the white spotted and purple color morphs lacked allele 5. In addition, at PGM-1, the purple spotted and purple morphs lacked allele 3. At PGM-2 and G3PDH-NADP-1 the white color morph showed no variation (all individuals were heterozygous) while individuals of the other three color morphs gave variable phenotypes, even though, on average the allele frequencies were similar.

Nei's genetic identities exceeded 90% for all color morphs (Table 3). Comparisons of purple spotted, white spotted, and purple color morphs all gave very high genetic identities ( $\bar{I} = 0.99$ ) whereas the white color morph consistently showed less genetic similarity to the other three morphs ( $\bar{I} = 0.94$ ).

Within population diversity ( $H_s$ ) accounted for 90.0% of the electrophoretic variation, while among population diversity ( $D_{st}$ ) accounted for 10.0% of the variation. For five of the polymorphic loci,  $G_{st}$  was significantly greater than zero based on chi square at  $\alpha = 0.05$  (Table 4).

### Morphology.

Petal length and width.—The petals of the white flowered *Clarkia* averaged 1.4 to 2.5 mm longer than those of the purple, purple spotted and white spotted flowers which had similar mean petal lengths and widths (ANOVA, Tukey test for pairwise comparisons involving the white morph at  $p < 0.05$ ; Table 5). The mean petal

TABLE 4. NEI'S STATISTICS OF GENE DIVERSITY AND DIFFERENTIATION AT THE EIGHT POLYMORPHIC LOCI. An asterisk (\*) in the  $G_{st}$  column indicates significant deviation from zero based on chi square.

|              | $H_i$ | $H_s$ | $G_{st}$ |
|--------------|-------|-------|----------|
| Est-2        | 0.376 | 0.301 | 0.200*   |
| Idh-2        | 0.382 | 0.388 | 0.000    |
| Aco-2        | 0.257 | 0.255 | 0.008    |
| Pgm-1        | 0.618 | 0.517 | 0.163*   |
| Pgm-2        | 0.500 | 0.511 | 0.000    |
| Aat-2        | 0.436 | 0.363 | 0.167*   |
| G3pdh-nadp-1 | 0.328 | 0.242 | 0.260*   |
| G3pdh-nadp-2 | 0.748 | 0.705 | 0.572*   |

width of the white color morph was also significantly larger than the other three morphs (ANOVA, Tukey at  $p < 0.05$ ; Table 5).

In the common garden (including the purple spotted, purple and white color morphs) the trends observed for field populations were maintained, although mean values were smaller (ANOVA,  $p < 0.05$ ; Table 5).

Leaf length and width.—The white spotted and purple color morphs did not differ significantly from each other in either mean leaf length or width while all other comparisons of the color morphs were significant (Table 5). The purple spotted morphs had wider leaves while the white flowered plants had longer leaves (Table 5). These differences in leaf morphology did not occur in the garden plants (ANOVA,  $p > 0.05$ ; Table 5).

Height and fruit length.—In both height and fruit length, the white morph differed significantly from all other morphs. Plants of this morph were twice as tall as the other morphs (ANOVA, Tukey at  $p < 0.05$ ; Table 5). Mean fruit length averaged 3.8–10.5 mm greater for the white morph than for the purple, purple spotted and white spotted morphs (Tukey at  $p < 0.05$ ) which were not significantly different from each other (Table 5).

Unfortunately, we were unable to measure height and fruit length in the common garden. Because the white morph is found in a different population from the other morphs, we cannot determine whether the observed height difference is a result of genetic differences between the morphs, environmentally induced differences, or some combination of the two. We could not effectively measure height in garden grown *Clarkia* due to the multibranching and more prostrate form of the garden plants. We presume that differences in plant form under field vs. garden conditions are related to greater light availability and the absence of competing vegetation in the garden. We did not measure fruit length because an inappropriate herbicide application led to plant death prior to fruit maturation.

#### DISCUSSION

A primary goal of this study was to assess the systematic and evolutionary implications of floral color variation in an endemic plant, *Clarkia amoena* var. *pacifica*. Our results provide evidence that both morphological and electrophoretic differences accompany petal color variation in coastal populations of this species.

In general, field measurements suggest that the white morph is larger than the other morphs. Lack of significant variation in leaf length and width in the garden grown plants indicates that leaf size plasticity in field grown plants may largely be a result of environmental factors. Differences in floral morphology observed in the field however, were maintained when plants were grown in the gar-

TABLE 5. MEANS AND STANDARD DEVIATIONS FOR MORPHOLOGICAL CHARACTERISTICS OF FIELD AND GARDEN PLANTS. An asterisk (\*) indicates  $P < 0.05$  based on ANOVA. Abbreviations as in Table 1. Different letters indicate groups which are significantly different.

| Char.             | WS                      | PS                      | P                       | W                       | F      | df  |
|-------------------|-------------------------|-------------------------|-------------------------|-------------------------|--------|-----|
| Petal width (mm)  | 9.2 ± 1.3 <sup>a</sup>  | 9.2 ± 1.9 <sup>a</sup>  | 9.5 ± 2.3 <sup>a</sup>  | 11.7 ± 1.4 <sup>b</sup> | 24.1*  | 196 |
|                   | —                       | 7.4 ± 0.9 <sup>c</sup>  | 7.8 ± 1.0 <sup>c</sup>  | 10.0 ± 1.0 <sup>d</sup> | 26.1*  | 37  |
| Petal length (mm) | 11.3 ± 1.5 <sup>e</sup> | 11.5 ± 1.8 <sup>e</sup> | 12.2 ± 2.3 <sup>e</sup> | 13.6 ± 2.1 <sup>f</sup> | 14.5*  | 196 |
|                   | —                       | 9.7 ± 0.9 <sup>g</sup>  | 10.4 ± 1.3 <sup>g</sup> | 12.5 ± 1.7 <sup>h</sup> | 14.5*  | 37  |
| Leaf width (mm)   | 5.3 ± 1.4 <sup>f</sup>  | 7.0 ± 1.3 <sup>f</sup>  | 5.5 ± 1.1 <sup>f</sup>  | 6.1 ± 1.3 <sup>h</sup>  | 18.4*  | 196 |
|                   | —                       | 6.0 ± 1.3 <sup>f</sup>  | 5.6 ± 0.9 <sup>f</sup>  | 5.0 ± 0.8 <sup>f</sup>  | 2.6    | 37  |
| Leaf length (mm)  | 22.4 ± 4.5 <sup>m</sup> | 25.7 ± 5.1 <sup>n</sup> | 23.4 ± 5.9 <sup>m</sup> | 34.3 ± 6.4 <sup>p</sup> | 48.3*  | 196 |
|                   | —                       | 19.3 ± 3.6 <sup>n</sup> | 21.1 ± 4.4 <sup>n</sup> | 18.2 ± 3.5 <sup>n</sup> | 1.9    | 37  |
| Height (cm)       | 9.7 ± 3.4 <sup>f</sup>  | 12.1 ± 4.1 <sup>f</sup> | 13.3 ± 0.8 <sup>f</sup> | 30.6 ± 9.4 <sup>f</sup> | 123.2* | 196 |
|                   | 20.1 ± 4.4 <sup>f</sup> | 24.8 ± 4.5 <sup>f</sup> | 26.8 ± 3.3 <sup>f</sup> | 30.6 ± 4.5 <sup>n</sup> | 13.2*  | 36  |

den under common conditions. This is significant because, in *Clarkia*, taxonomic separations are often based entirely on floral characters. In fact, many *Clarkia* species could not be differentiated if stripped of their flowers (MacSwain et al. 1973).

Overall, statistics of genetic diversity in *Clarkia amoena* var. *pacifica* indicate higher within than among population diversity. Among population diversity ( $D_{st}$ ) accounts for 10.0% of the variation observed; the value for  $G_{st}$  is within the range of values considered indicators of moderate genetic differentiation (Hartl 1988). On average, plant species with a mixed pollination system have a  $G_{st}$  value of 0.21 (Hamrick et al. 1991). Although the  $G_{st}$  found in this study ( $G_{st} = 0.10$ ) is somewhat lower than the average, there is still some difference among the populations.

Although no alleles were unique to a particular color morph, the white morph lacked an allele for AAT-2 that was present in all other color morphs. In addition, for two loci, PGM-1 and G3PDH-NADP-2, the white differed from the other morphs in the most frequently occurring allele. Also, the white color morph lacked variation in both PGM-2 and G3PDH-NADP-1 (it was heterozygous for all individuals) while the other color morphs were variable.

Results of electrophoresis also revealed that the white color morph shows a slightly reduced identity relative to the other more genetically similar purple and white spotted and purple morphs. On average, conspecific populations of plants have a mean genetic identity of 0.95 (Crawford 1983). In *Clarkia*, reproductively isolated populations of a single species, *C. xantiana*, had relatively high genetic identities of .91 and .89 (Gottlieb, 1984) whereas comparisons of two species, *C. speciosa* and *C. nitens* gave an identity of .94 (Soltis and Bloom 1991). Therefore, in *Clarkia*, high genetic identities between populations should not be taken as prima facie evidence that two populations are part of the same species. However, the level of genetic identity found between morphs in the two populations at Cascade Head ( $\bar{I} = 0.94$ ) is certainly equivalent to levels found among distinct species in *Clarkia* and lower than levels found among co-occurring color morphs ( $\bar{I} = 0.99$ ) within a single population. Thus, current levels of genetic differentiation indicate the potential for future speciation. The rapid evolutionary processes known in the genus also support the possibility that the two populations at Cascade Head may be in the initial stages of divergence.

Some pollinator and phenological differentiation are also present between the two populations. Based on field observations, the two populations have slightly staggered blooming times; the white population blooms later. In addition, the white color morph receives over one-third of its pollinator visits from vectors which have not been observed visiting the other colors (Foust and Butler pers. obs.). It is possible that these differences could be attributed to local environmen-



tal effects that influence pollinator diversity in the two populations. At Cascade Head however, there is a greater diversity of pollinators in the "pinnacle" region where the mixed population of *Clarkia amoena* var. *pacifica* occurs (Kephart personal communication), whereas we found a greater diversity of pollinators visiting the population of white morphs (Foust and Butler 1996). Additionally, most of the pollinators found visiting *Clarkia amoena* var. *pacifica* are small (Foust and Butler 1996) and are likely to have relatively short flight distances (Waser 1982). Although recent studies indicate that gene flow can occur over distances of greater than 0.5 km (Kirkpatrick and Wilson 1988; Broyles and Wyatt 1990), it is possible that the two populations of *C. amoena* var. *pacifica* are isolated from each other due to the differences in pollinators and blooming times.

Further studies of the color morphs including examination of chromosomal arrangements and hybridization experiments would provide more definitive evidence regarding the relationships of the populations of *C. amoena* var. *pacifica*. Frequent chromosomal rearrangements have occurred in this genus and the resulting arrangements have been used to differentiate species and subspecies (Lewis 1953; Gottlieb 1973). Knowledge of chromosomal arrangements along with the morphological and genetic differences found here could provide convincing evidence that speciation is occurring between the two populations of *Clarkia amoena* var. *pacifica*.

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